Faculty of Resource Science and Technology

GRACILARIA SP. IN ASAJAYA MANGROVE WITH SPECIAL REFERENCE TO ITS PRODUCTION

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the requirement for the degree of Bachelor of Science with Honours
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Gracilaria sp. in Asajaya Mangrove Area with Special Reference to Its Production

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ABSTRACT

This study aimed to record the biomass and identifies the species of *Gracilaria* that inhabit the Asajaya mangrove area. The Asajaya mangrove has been divided into three stations and Station 3 was further divided into five permanent plots. The samples of Gracilaria sp. were collected monthly from September to December 2009 at the five permanent plots. Taking the tree of *Sonneratia alba* as a centre, *Gracilaria* was collected around the tree with radius of 10 m from the centre. The samples were dried in oven for 48 hours at 60°C and weighed to obtain the dry weight. *Gracilaria changii* is the species that has been identified in this study. The temperature in Asajaya mangrove ranged from 27°C to 30.2°C. The pH was recorded as alkaline which ranged from 7.54 to 7.66. For salinity, it ranged from 20.3 PSU to 21.9 PSU. Meanwhile dissolved oxygen (DO) ranged from 6.7 mg/L to 9.7 mg/l. The biological oxygen demand in 5 days (BOD_5) recorded the lowest value in Station 1 with 7.08 mg/l and the highest in Station 2 with 10.63 mg/l. Biomass (g dry weight/m^2) was calculated for each subplot and its ranged from 0.04 to 0.21 g/m^2 DW. The agar yield without alkali treatment is 15 ± 0.77% and 17 ± 0.85% for alkali treatment.

Key words: *Gracilaria* sp., biomass, agar

ABSTRAK

Kajian ini bertujuan untuk merekod biomass dan mengenal pasti spesies Gracilaria *yang wujud di kawasan paya bakau Asajaya. Paya bakau Asajaya telah dibahagikan kepada 3 stesen dan Stesen 3 dibahagikan selanjutnya kepada 5 plot kekal. Penuaian* Gracilaria *sp. telah dibuat pada setiap bulan bermula September hingga Disember 2009 di 5 subplot yg telah ditetapkan. Dengan pokok* Sonneratia alba *sebagai pusat,* Gracilaria *dituai di sekitar pokok tersebut dengan jejari 10 m daripada pusat.Sampel dikeringkan di dalam ketuhar selama 48 jam pada suhu 60°C dan ditimbang untuk mendapatkan berat kering.* Gracilaria changii *adalah spesies yang dikenal pasti dari kajian ini. Suhu paya bakau Asajaya adalah di dalam julat 27°C ke 30.2°C. pH yang direkod adalah alkali di dalam lingkungan 7.54 ke 7.66. Untuk paras kandungan garam, ia berada di dalam julat 20.3 PSU ke 21.9 PSU. Manakala oksigen terlarut (DO) berada dalam julat 6.7 mg/L ke 9.7 mg/l. BOD_5 direkodkan terendah di Stesen 1 dengan nilai 7.08 mg/l dan tertinggi di Stesen 2 dengan nilai 10.63 mg/l. Biomass (g/m^2 berat kering) dikira untuk setiap subplot dan di dalam lingkungan 0.04 to 0.21 g/m^2 DW. Hasil agar tanpa rawatan alkali ialah 15 ± 0.77% and 17 ± 0.85% untuk rawatan alkali.*

Kata kunci: Gracilaria *sp., biomass, agar*

1.0 INTRODUCTION

Seaweed can be defined as macroscopic marine algae from the division of Chlorophyta (green algae), Rhodophyta (red algae) and Phaeophyta (brown algae) (Ahmad, 1995). This macroscopic marine alga can be seen with naked eye or at the most with the help of low-power hand lens of 10x magnification (Teo & Wee, 1983). They inhabit the benthic division or the sea floor and also called as attached algae (Abott & Dawson, 1978). The attachment of seaweeds is by a structure called holdfast, a simple modified portion of plant body (Teo & Wee, 1983).

Mangroves are woody plants that grow at the interface between land and sea in tropical and sub-tropical latitudes where they exist in conditions of high salinity, extreme tides, strong winds, high temperatures and muddy, anaerobic soils. The woody plants associated with microbes, fungi, plants, and animals, constitute the mangrove forest community or mangal.

The mangal and its associated abiotic factors constitute the mangrove ecosystem. Macnae (1968) proposed that "*mangal"* should refer to the forest community while "mangroves" should refer to the individual plant species. Mangrove forest that usually found near the coastal water, estuaries and around the island is a group of plants that have several unique characteristics that combined the characteristic of plant that live in the land and sea (Kanthaswamy *et al*., 1994)

The macroalgal flora in mangrove habitats contributes to production, providing habitat and food for a number of invertebrate and fish species. The genera *Bostrychia*, *Caloglossa* and *Catenella*, of the red algae are most commonly associated with mangroves and may be quite abundant (Kathiresan and Bingham, 2001). Algal assemblages tend to be richest in shallow areas with a mixture of hard and soft substrates. Lowest diversity occurs where there is low light, soupy muds, or homogeneous, large-grain sands (Kathiresan and Bingham, 2001).

Algal abundance and diversity are largely determined by the physico-chemical characteristics of the mangal. As with the mangroves themselves, the most successful macroalgae have special adaptations that help them tolerate extreme conditions. Salinity, temperature, desiccation, tidal inundation, wave action, wetting frequency and light intensity are all environmental factors likely to produce patterns of horizontal and vertical distribution seen in many mangrove algae (Kathiresan and Bingham, 2001).

The objectives of this study are to record the biomass index of *Gracilaria* in the Asajaya mangrove and to extract agar by using native and alkali treatment.

2.0 LITERATURE REVIEW

2.1 Family Gracilariacea

Gracilaria is the most significant genus in the family of Gracilariacea in term of number of species and distribution throughout the world. The characteristic that shared among the family members is the carposporophyte developing toward the outside of the thallus, cruciately divided tetrasporangia, and a pseudoparanchymatous construction such that the cells of the medulla are more or less isodiametric rather than filamentous (Sze, 1993). The taxanomic hierarchy of the genus is as follows:

Phylum: Rhodophyta

Class: Rhodophyceae

Order: Gigartinales

Family: Gracilariaceae

Genus: *Gracilaria*

2.2 Distribution

2.2.1 Distribution of *Gracilaria* in Malaysia

From part of the chapter on the seaweed resources of Malaysia in 'Seaweed Resource of the World', Phang (1988) has recorded 260 specific and infraspecific taxa (17 cyanophyta, 92 chlorophyta, 94 rhodophyta and 57 phaeophyta). Among the tropical seaweed flora, Rhodophyta dominated. The rhodophyta are filamentous and comprise of epiphytic species.

The red seaweeds have the highest number of taxa. *Halymenia,* the large foliose species dominate the subtidal bedrock area, while *Laurencia* and *Hypnea* species, the proliferous branching thalli inhabit the bedrocks at the intertidal regions and grow mainly in the cleaner deep waters. Species of *Eucheuma* and *Kappaphycus* can be found from lower intertidal to upper sub-tidal areas in Sabah and around islands in Peninsular Malaysia. Lim and Phang (2004) stated that there are twenty (20) species of the agarophytic genus *Gracilaria* have been reported (refer Table 1), many of which inhabit mangroves, sandy-mudflats and rocky shores. Rhodophytes are commonly found in the coral reefs especially in the cleaner deep waters around the islands (Phang, 2006).

Table 1: Distribution and habitat of species *of Gracilaria* in Malaysia

Species of *Gracilaria*	Distribution	Habitat
G. articulate	Peninsular Malaysia	Mud
G. canaliculata	Peninsular Malaysia	Coral, Mud, Sand
G. blodgetti	West Coast Peninsular Malaysia	Mud
G. cacalia	Singapore	Coral
G. changii	West and East Coast Peninsular Malaysia	Mangrove, Mud, Rock, Sand
G. coronopifolia	West and East Coast Peninsular Malaysia, Singapore	Coral, Mud
G. crassa	Sabah, Singapore	Driftweed
G. dura	Sabah, Singapore	–
G. edulis	West Coast Peninsular Malaysia, Sarawak, Singapore	Mud, Rock, Sand
G. eucheumoides	Peninsular Malaysia	–
G. firma	West Coast Peninsular Malaysia, Sabah	Rock
G. folifera	West Coast Peninsular Malaysia	Rock
G. lichenoides	Peninsular Malaysia	–
G. manilaensis	Peninsular Malaysia, West Coast Peninsular Malaysia	Mud
G. salicorna	West and East Coast Peninsular Malaysia, Sabah	Mangrove, Mud, Rock, Sand
G. subtilis	West Coast Peninsular Malaysia	Sand, Mud
G. tenuistipitata	West Coast Peninsular Malaysia	Rock
G. textorii	West Coast Peninsular Malaysia	Rock
G. urvillei	West Coast Peninsular Malaysia, Sabah, Singapore	Sand, Mud
G. verrucosa	Peninsular Malaysia,Sabah	–

Source: Phang (2006).

2.2.2 Distribution of *Gracilaria* in Sarawak

Sarawak has a land area of 124 449 km^2 which covers 38% of Malaysia's land mass with a coastline length approximately 1000 km. Climate along the Sarawak Coast is tropical monsoon with high temperature, high humidity, and heavy seasonal rainfall. The average daily temperature along Sarawak coast is range from 22-33°C, with very little seasonal variation. Rainfall shows seasonal variation in respond to the monsoon periods. Sarawak coast have large tidal range, up to 6 meters. The coastal waters are enriched with nutrient leached which can support a highly diverse flora including the seaweeds and fauna (Han, 2007). The table below show the species of *Gracilaria* that can be found in Sarawak.

Table 2: Distribution and ecology of *Gracilaria* in Sarawak.

Species	Local Distribution	Ecology
Gracilaria arcuata	Pantai Similajau, Bintulu Divisi	Attached to corals or rocks. Grows in shallow, subtidal area attached to corals
Gracilaria changii	Pulau Salak, Kuching Division	Attached to net, buoys and floating net cages
Gracilaria coronopifolia	Pulau Salak, Kuching Division	Attached to nets, buoys at floating net cages systems
Gracilaria blodgetti	Muara Mengkuang, Miang Kecil, Sungai Sibu, Salak, Kuching Division	Attached to mangrove roots
Gracillaria salicornia	Pantai Similaju, Bintulu Division	Attached to rock, numerous in pool between high-low tide zones

Source: Han (2007)

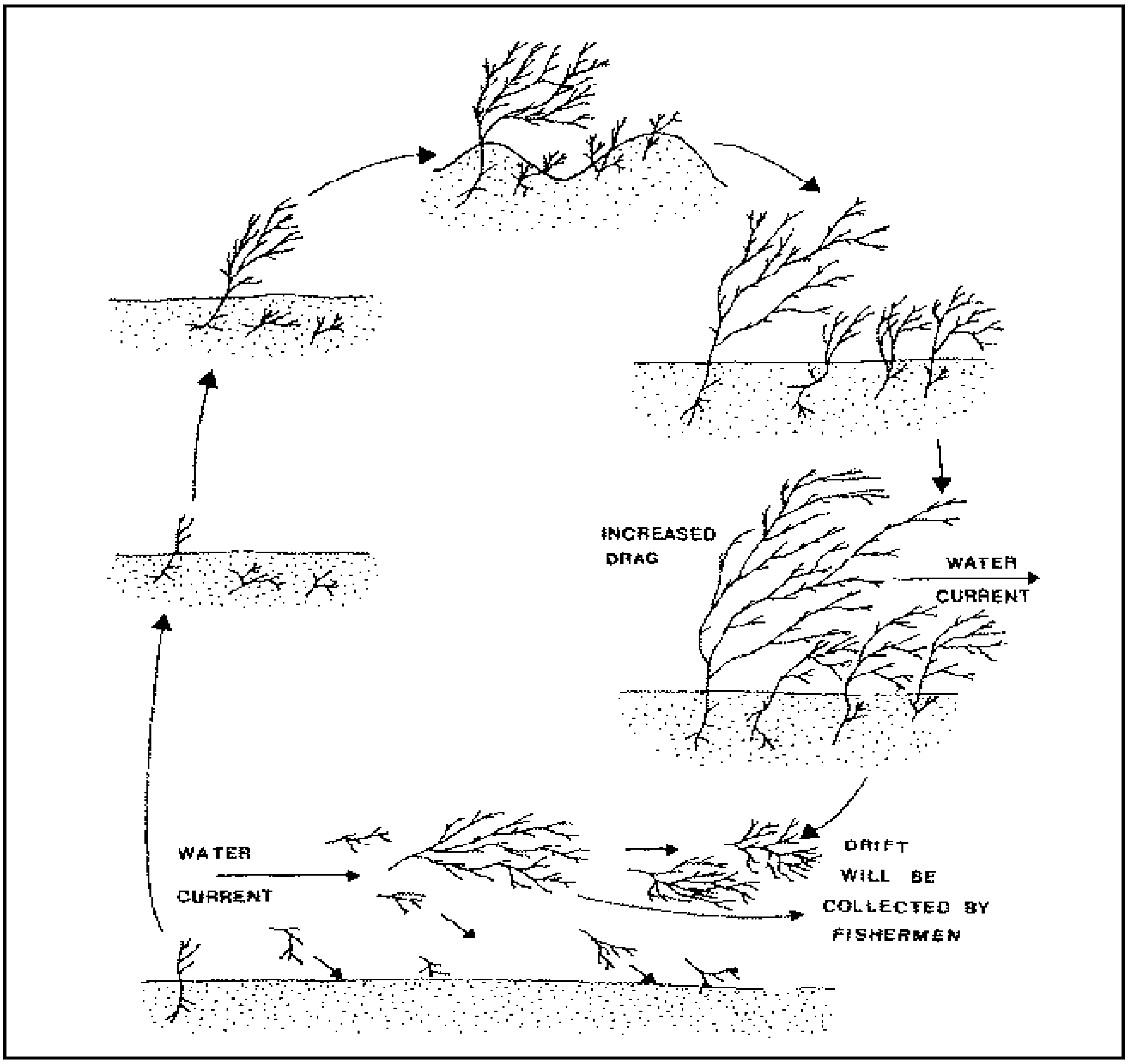
2.3 Local Species

According to the Final Year Project report by Ariffin (2005) on seaweeds community in Asajaya mangrove, there are five taxa with two species from the genus *Gracilaria* and one each from the genus *Catanella*, *Laurencia* and *Enteromorpha*. *Gracilaria changii* was abundant in the low tide zone and attached to the pneumatophores of *Sonneratia alba* and *Avicennia alba* and often submerge in the tide pool. *Gracilaria edulis* can be found in the low-tide and inter-tidal zone associated with *Sonneratia* and *Avicennia*.

2.4 Ecological Aspects of Seaweeds

2.4.1 Natural sediment habitat of *Gracilaria*

The sandy or muddy bottom relief changes due to water movement and some of these thallus fragments become uncovered and start growing. As underground thalli are exposed to light, they start growing. They will grow and branch until their size and shape lead to increases in frictional drag. Portions of these plants are removed by water movement. A fraction of these materials will sink, eventually becoming covered by sand and giving rise to more underground thalli (refer Diagram 1). (Santelices & Doty, 1989)



Source: Santelices & Doty (1989)

Figure 1: Diagram of the natural sediment habitat of *Gracilaria* showing the dynamic of underground and emerging thalli.

2.4.2 Biotic Interactions

Competition within and between species for space, light, nutrients and any other limiting resource was included in this interaction. Seaweed species are sometimes competing amongst each other for space. Fish, sea urchin and mesograzers are the three common groups of herbivores that dwell in seaweed community. Impact of herbivores on seaweed population can cause great destruction. Example of grazing is the seaweed-urchin-predator interactions (Lobban & Harrison, 1994).

2.4.3 Light and Photosynthesis

In the photosynthesis process, light is absorbed by pigments, convert into chemical energy in the form of ATP (adenosine triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate) that will be used in the synthesis of organic compounds from CO_2. Photosynthetically Active Radiation (PAR) is usable light for photosynthesis that occurs in the range from 400-700 nm of the electromagnetic spectrum. Accessory pigment absorb different wavelength of PAR and transfer the light energy to chlorophyll *a*. the photosynthetic pigments are associated with phycobiliprotein in the thylakoid membranes to form light-harvesting complex. The principal photosynthetic pigments of the rhodophyta are chlorophyll *a* and phycoerythrobilin (Sze, 1986).

2.4.4 Nutrients

Seaweeds require various essential elements (nitrogen, phosphorus, calcium, magnesium, copper, zinc) and up to three vitamins (Vitamin B_{12} or cyanocobalamin, thiamine, and biotin) for growth. Concentration of elements such as nitrogen, phosphorus and iron may be low enough to limit growth of seaweeds. (Lobban & Harrison, 1994)

2.4.5 Temperature and Salinity

The surface temperatures of seawater vary with latitude and ocean currents. Biochemical reaction rates double for every 10°C rise in temperature. Photosynthesis, respiration, growth, and enzyme reactions have their own optimum temperature but the effects of temperature on all these process are not uniform. Natural salinities in marine and brackish waters range from 10 to 70‰. In intertidal seaweeds, there are extreme salinity changes due to evaporation, rain or runoff. The total concentration of dissolved salts and the corresponding water potential, plus the availability of calcium and bicarbonate are the important components of salinity (Lobban & Harrison, 1994).

2.4.6 Effect of Salinity and Temperature on Photosynthetic and Growth Response

Salinity is one of the chemical factors that affect the distribution of seaweed. The population of *Gracilaria salicorna* at Phuket Island and Rayong, Thailand had a euryhaline photosynthetic response between 20 to 33 psu. The photosynthetic response under hyposaline treatment caused a reduction in maximum photosynthesis of the *G. salicorna*. Populations from Thailand had higher photosynthetic potential under conditions of high irradiance and high temperatures. Such characteristics in photosynthesis and respiration may be related to the environmental conditions of their natural habitats, as Thailand is located near the equator with tropical climate. (Phooprong, 2007)

2.5 Importance of Seaweeds

2.5.1 Food Source

The edible seaweeds of the red algae are *Porphyra, Palmaria, Gracilaria, Gelidium and Eucheuma*. People living in the coastal areas consumed the fresh and dried seaweeds. Seaweeds as a food in Malaysia is not as common as in countries like Japan and China. 25% of all food consumed by the Japanese consist of seaweed prepared and serve in many forms such as the *Porphyra* that is used as wrapper for sushi. However in Malaysia, the seaweed is only consumed in coastal areas especially along the east coast of Peninsular Malaysia and in East Malaysia as a salad dish (Hani and Ching, 2000).

2.5.2 Sources of hydrocolloid

In North America, seaweeds are used as source of hydrocolloid rather than direct consumption. Hydrocolloid is derived from the mucilage associated with the cell walls. Hydrocolloids that derived from the red algae are carageenan and agar. The sources of agar are from *Gelidium* and *Gracilaria*. Important uses of agar are as food gels, medium for culturing microorganisms and binder for medical tablets and capsules. Important commercial sources of carageenan are *Chondrus, Gigartina* and *Eucheuma*. The major industrial uses of carageenan are air freshener gels, thickening agent and emulsifier (Sze, 1993).

2.5.3 Ecological Importance

Seaweeds together with the seagrass stabilize sediments and prevent erosion along the coastline. Seaweeds community provides habitat for the small protist, invertebrate and other small algae. Their leafy form provides shelter for the organisms from predator and heavy waves. Seaweed that decayed on the seaside will produce stink smells. During this condition, the decayed seaweeds and the stink smells will attract many kinds of insect especially fly. Organisms that ate and sheltered on the decayed seaweeds will be as food for other organisms (Ahmad, 1995).

2.5.4 Biofilters

Seaweeds have been integrated into fish farming as the solution to the problem of heavy nutrient loads discharge into coastal water from an intensive fish farm since seaweeds can removed 90% of the nutrients. Therefore, algal farming along the coasts could be an effective biofilter to alleviate the eutrophication problem (Luning and Pang, 2003). *Gracilaria* that co-cultivated with salmon in a tank system could remove 50% of the dissolved ammonium released by the fish in winter, increasing to 90–95% in spring and assimilated 6.5% of the released dissolved nitrogen (Troell *et al*., 1999).

2.6 Nutritive value of seaweeds

Worldwide, around 221 seaweed species belonging to 32 Chlorophyta, 64 Phaeophyta and 125 Rhodophyta are being used for variety of purposes. Of these, about 145 species (66%) are used for food (Zemke-White and Ohno, 1999). From a nutritional point of view, edible seaweeds are low calorie food, with a high concentration of minerals, vitamins and proteins and a low content in lipid. Seaweeds are excellent source of vitamins A, B1, B12, C, D and E, riboflavin, niacin, pantothanic acid and folic acid as well as minerals such as Ca, P, Na, K (Dhargalkar & Verlecar, 2009). The important edible seaweed genera are Porphyra, Gracilaria, Laurencia, Enteromorpha, Monostroma, Caulerpa and etc. Hani & Chio (2000) analyzed *Gracilaria changii* found in Malaysia for its biochemical and mineral composition. It was found to contain 6.9% protein level (wet weight basis) which is lower than in soybeans

(33.8%), slightly higher than in peas (3.4%) and broccoli (4.1%) but much higher than vegetables such as carrot, tomato and cabbage. The fibre content in *G. changii* which is found to be 24.7% is about 5 time higher fibre content than other vegetables. It was also found that vitamin C content in *G. changgi* (28.5%) is comparable to that in lettuce and tomato. *G. changii* is also rich in omega fatty acid which is rarely found in vegetables.

2.7 Agar properties

Agar is a polysaccharide extracted mainly from members of Gelidiales and Gracilariales and use in food industries because of its gelling strength (Teo & Wee, 1983). It has linear structure of repeating units of the disaccharide agarobiose, a dimmer of D-galactose and 3,6-anhydro-L-galactose. The common extraction process of agar is obtained by leaching of the alga in hot water, filtering off the extract, and separating the agar by freezing and thawing to eliminate the water. Pre-treatment with alkali (NaOH) is needed for the desulphation of the native agar, causing the formation of a 3,6-anhydrogalactose bridge and an increase in the gel strength of the alkali treated agar (Arvizu-Higuera *et al*., 2007). Agar yield range from 10.8 to 25.12% dry weight whereas gel strength ranged from 293.65 to 562.52 g cm^{-2}. Melting temperature of *Gracilaria changii* ranged from 83 to 89°C while gelling temperature ranged fron 32 to 39°C (Phang *et al.*, 1996).

Table 3 shows 27 species of *Gracilaria* that summarize the importance and use of the species worldwide. They are commonly use for food, agar, carageenan, alginate, medicine and paper.

Table 3: Algal species utilised world-wide, country and uses. F = food, A = agar, C = carrageenan, Ag = alginate, M = medicine, P = paper

Species of *Gracilaria*	Use	Country
Gracilaria sp.	Ag C F P M	Portugal Malaysia Myanmar, Thailand Italy Vietnam
Gracilaria asisatica	A F	China, Vietnam Vietnam
Gracilaria bursa-pastoris	F	Japan
Gracilaria caudata	A	Brazil
Gracilaria chilensis	A Ag	Chile New Zealand
Gracilaria cornea	A F	Brazil Caribbean
Gracilaria coronopifera	F	Hawaii, Vietnam
Gracilaria crassissima	F	Caribbean
Gracilaria domingensis	F	Brazil, Caribbean, Chile
Gracilaria edulis	A	India
Gracilaria eucheumoides	F M	Indonesia, Vietnam Indonesia
Gracilaria firma	A C F	Philippines, Vietnam Philippines Vietnam
Gracilaria fisheri	A F	Thailand
Gracilaria folifera	A	India
Gracilaria gracilis	A	Namibia, South Africa
Gracilaria heteroclada	A	Philippines

Cont.

	F	Vietnam
Gracilaria howei	A	Peru
Gracilaria lemaneiformis	A F	Mexico, Peru Japan
Gracilaria longa	A	Italy
Gracilaria pacifica	A	Canada
Gracilaria parvispora	F	Hawaii
Gracilaria salicornia	A F	Thailand Thailand, Vietnam
Gracilaria tenuistipitata var. liui.	A F	China, Philippines, Thailand, Vietnam Thailand, Vietnam
Gracilaria verrucosa	A F M	Argentina, Egypt, Italy France, Indonesia, Japan , Korea Indonesia
Gracilariopsis lemaneiformis	A	Canada
Gracilariopsis tenuifrons	A	Brazil

Source: Zemke-White & Ohno (1999)

3.0 METHODOLOGY

3.1 Study Site

Asajaya mangrove area was selected for study site. The sampling trip was conducted from September to December 2009. Three stations were established based on location and site characteristic. Station 1 for rocky shore, Station 2 for middle section and Station 3 for seaward mangrove area. Station 3 was subdivided into five permanent plots for *Gracilaria* sampling. This is because there were abundant of *Gracilaria* growing at the Station 3 while very scarce at the other stations. Taking the tree of *Sonneratia alba* as a centre, *Gracilaria* was collected around the tree with radius of 10 m from the centre. GPS (Global Positioning System) was used to coordinate the location of the sampling stations.

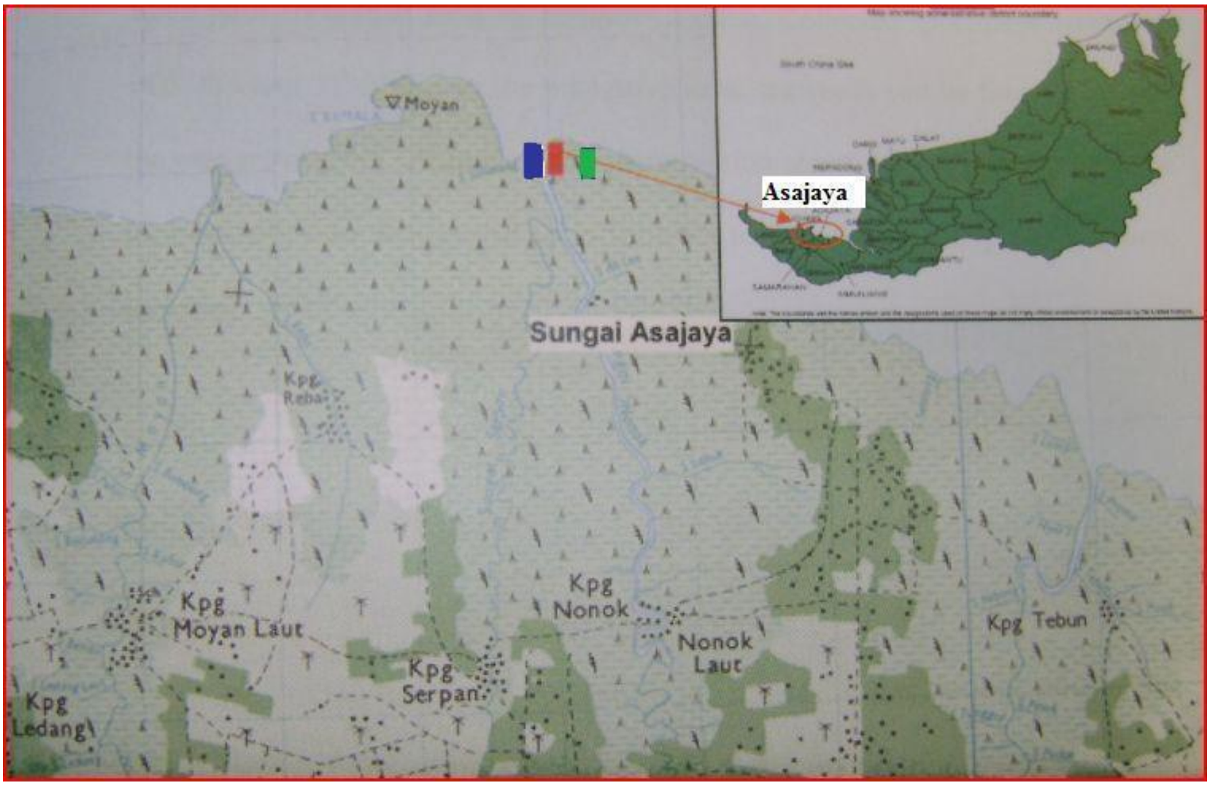


Figure 2: Study site. Site 1 Site 2 Site 3

Table 4: GPS reading for each sampling stations

Station	Description	GPS reading
1	Rocky shore	N 01° 35’ 51.8” E 110° 36’ 02.1”
2	Mangrove (middle section)	N 01° 36’ 04.1” E 110° 35’ 45.0”
3	Mudflat (seaward)	N 01° 36’ 03.0” E 110° 34’ 36.5”

3.2 Physico-chemical Parameters

Measurements of physico-chemical parameters were taken *in-situ* at the 3 stations. Temperature and pH reading were measured using Hanna pH/ORP meter. Dissolved oxygen (DO) was measured using DO meter while salinity was measured using hand refractometer.

3.3 Sample Collection

Tools for collecting samples include pail, plastic bags and label, and putty knife. The whole part of sample including the holdfast was taken out form substrate. Then, the specimen was kept in plastic bags. Each plastic bag was labelled. Other information such as date, location, type of substrate, were recorded. Samples were rinsed with seawater to remove debris and epiphyte.

3.4 Laboratory

The samples were stored in the cooler box when brought back from the sampling site to the laboratory and were kept in the freezer before processing. All samples were defrosted and rinsed in fresh water to remove sand, silt, epiphytes and other debris. Samples were dried for at least 48 hours at 60°C until the dry weight was constant.

3.5 Biomass

Biomass of *Gracilari*a sp. for every station was calculated using the following formula:

$$\boxed{\frac{\text{Dry weight (g)}}{\text{Area (m}^2\text{)}}}$$

* Area $= \pi r^2$

$= 3.142(10)^2$

$= 314.2\ m^2$

3.6 Biological Oxygen Demand in 5 days (BOD_5)

Water sample were collected in BOD bottle and ensure no bubbles were trapped. DO reading was recorded *in-situ*. The bottle was wrapped with aluminium foil to avoid direct sunlight. The bottles were placed in the room temperature (25°C). The DO reading was recorded again after five days. BOD_5 (mg/l) was calculated using the following formula:

$$\boxed{\frac{\text{initial } \textit{in-situ} \text{ DO reading - DO reading on day 5}}{\text{volume of water sample (l)}}}$$

3.7 Nutrient Analysis

The nutrients included in this analysis were nitrate, ammonia-nitrogen and orthophosphate. The water samples for these analyses were filtered using filtering system with glass microfiber filter size 47 mm to maximize the purity of the water sample and to evacuate the sand and other materials that can obstruct the analysis.